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"Gene Diagnosis—Introduction of New Technology" : The Tenth International Symposium of the Hiroshima Cancer Seminar, October 2000

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The Tenth International Symposium of the Hiroshima Cancer Seminar (HCS) Foundation was held on 29 October 2000 at the International Conference Center, Hiroshima. The symposium consisted of seven special lectures; about 170 people were present and there were presentations and discussions on gene diagnosis. For the Open Lecture to the public on 28 October 2000, Ichiro Tsuji (Tohoku University, Sendai) and Tetsuichiro Muto (Cancer Institute Hospital, Tokyo) spoke about the mechanisms of cancer development, prevention and treatment to more than 250 people.

At the beginning, Wataru Yasui (Hiroshima University, Hiroshima), Chairman of the Organizing Committee of the Tenth International Symposium, and Executive Director of the HCS Foundation, gave an opening address. Yasui gave a brief background to this series of symposia. The HCS Foundation was established in 1992 with the cooperation of Hiroshima Prefectural Government, Hiroshima Financial Circles, Hiroshima Medical Association and Hiroshima University, and holds an annual international symposium. The symposium is organized to create an opportunity for basic scientists and clinical researchers to exchange ideas on cancer research, cancer prevention and cancer therapy. This year, the organizing committee planned to explore the very recent and important issue of Gene Diagnosis—Introduction of New Technology. With the sequencing of the human genome, vast information on the molecular pathogenesis of diseases will become available. By applying new technology such as cDNA microarrays, the quality of gene diagnosis can be improved, leading to huge advances in personalized medicine and evidence-based medicine.

Special lectures on gene diagnosis

Dr. John Quackenbush (The Institute for Genomic Research (TIGR), Maryland, USA) opened the symposium by describing whole-genome functional analysis of human colon metastasis using cDNA microarrays. A goal of the Human Genome Project is identification of the complete set of human genes and the roles played by these genes in development and diseases. Microarrays provide the opportunity to study gene expression patterns on a genomic scale. Thousands of cDNA clones are arrayed on a microscope slide and relative expression levels of the genes are determined by measuring the fluorescence intensity of labeled mRNA hybridized to the arrays. The data provided by microarray analysis promise to yield functional information on a genomic scale, allowing a significant fraction of the genes to be assayed in a single experiment. Further, this approach provides a means of identifying candidate genes that may play a role in human cancer development and progression. Cancer metastasis is primarily a result of altered gene expression. Using cDNA microarrays, a study of gene expression changes in colon cancer metastasis has been initiated with the goal of identifying genes that are prognostic of metastasis. His group has assembled a collection of cDNA clones representing more than 40 000 distinct genes, developed laboratory hardware and protocols, and created databases and data analysis tools necessary to analyze differential expression. High-density cDNA microarrays have been used to study differential expression patterns between colon cancer cell lines of low metastatic (KM12C; SW480) and high metastatic (KM12L4A, KM12SM; SW620) potential. Statistical analysis of measured expression ratios suggests genes that may be of prognostic or diagnostic value and provides a more complete understanding of gene function and regulation with respect to cancer metastasis. A similar method can be utilized to analyze clinically derived samples. In order to apply microarray technology to human tumor specimens, rigorous and stringent protocols will need to be designed to ensure that the data gathered properly reflect gene expression in tissues. The degradation of RNA in tissue samples may have a significant effect on the expression measurements. A microarray comparison of RNA levels between identical samples subjected to different periods of ischemia could lead one to the incorrect conclusion that some of the genes on the array were differentially expressed. An investigation has been performing to examine how tissue handling affects RNA degradation and the ability to generate meaningful expression data using microarrays. The preliminary data show that temporal changes in gene expression levels do occur following tissue excision, with detectable changes after as little as 5 min.

Tatsuhiko Tsunoda (Institute of Physical and Chemical Research, Tokyo) and Yusuke Nakamura (University of Tokyo, Tokyo) described the prediction of sensitivity of esophageal tumors to anti-cancer drugs by cDNA microar-

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ray analysis of gene-expression profiles. In spite of recent progress in understanding the molecular genetics and biology of cancer, a large number of patients still suffer from side effects of anti-cancer drug therapy without experiencing any benefits. Various approaches have been attempted to permit prediction of sensitivity or resistance to adjuvant therapy. Since the properties of cancer cells can vary enormously from one patient to another, it is not possible to characterize individual tumors by means of a single, or even several, molecular markers, including multi-drug resistance genes. The development of sophisticated microarray technology has enabled us to analyze thousands of genes in a single experiment. The identification of dozens or hundreds of determinant genes for response to anti-cancer drugs might permit us to establish a novel diagnostic approach for personalized treatment of each cancer patient. The majority of esophageal cancers are squamous cell carcinomas that in general are more sensitive to chemotherapy and radiation than adenocarcinomas. In clinical trials, adjuvant and neo-adjuvant chemotherapies for patients with esophageal squamous cell carcinoma have appeared to improve survival times. However, a large proportion of patients suffered from severe side effects, and in some cases the drugs showed little or no effect on cancer cells. It is a matter of urgency to establish a diagnostic method to determine the precise properties of each cancer, including its metastatic ability and its sensitivity to chemotherapy and/or radiation. cDNA microarrays were applied to clarify the properties of esophageal cancers and to establish a genetic method for predicting the response of individual patients to anti-cancer drugs. The expression of 9216 genes was analyzed in 20 esophageal-cancer tissues from surgical patients who were treated postoperatively with the same adjuvant chemotherapy, and classified according to the duration of survival after surgery: over 30 months (group 1: 8 cases), 12–24 months (group 2: 6 cases), shorter than 12 months (group 3: 6 cases). By comparing the expression profiles of primary cancer tissues, his group developed a "drug response score (DRS)" on the basis of differential expression of these 52 genes and found a significant correlation between DRS and individual patients' prognoses. Validation with a further 4 patients (test cases) has supported the idea that DRS correctly predicts the prognosis of those patients. These results indicated that this scoring system, based on microarray analysis of selected genes, is likely to have great potential for predicting the response of individual cancer patients to chemotherapy. He also demonstrated the gene expression profiles of cancers of the colon, ovary and liver.

Masahiko Nishiyama (Hiroshima University, Hiroshima) described individualization of anticancer chemotherapy according to the molecular signature of tumors. Personalized medicine is a new drug-treatment strategy based on the idea that an individual's genetic make-up is directly related to the response to drugs. Genetic markers could be used either for screening out individuals with genotypes that cause severe toxicity or for selecting patients who are more likely to respond well. DNA microarray technology presents an opportunity not only to rapidly genotype individuals to provide information on polymorphic drug metabolism genes, but also to identify genes differently expressed in response to a drug. To determine the significant genetic markers for drug response, the expression patterns of approximately 20 000 genes were investigated in 10 human gastrointestinal cancer cell lines using DNA microarrays. The database will help in the search for novel genetic response markers to a variety of anticancer drugs in gastrointestinal cancers through the comparative analysis of drug sensitivity. He has established a treatment model focusing on the sensitivity determinants of cancer cells to drugs. A total of 25 factors related to cellular sensitivity (or resistance) to drugs were investigated to identify the most potent marker of anticancer activity, seeking the best modality of enhancing drug action in relation to the biochemical heterogeneity of cancer cells. Those approaches provided a unique anticancer chemotherapy model for gastrointestinal cancer patients, in which the best therapy was selected from 12 regimens according to the individual molecular signature of tumors based on 7 critical drug action determinants such as glutathione S-transferase, dihydropyrimidine dehydrogenase, thymidylate synthase (TS), and NADPH/quinoneoxidoreductase. Each chemotherapy selected in the system was a molecular target therapy, which could enhance drug activity by modulating the sensitivity or resistance determinants to the drugs. The relative predominance of these drug-action determinants can be demonstrated by gene expression analysis, thus aiding in selection of the most active forms of mitomycin C (MMC), 5-fluorouracil (5-FU), cisplatin (CDDP) and Docetaxel (TXT) treatment. A clinical trial is now going on, and a good anticancer effect has been obtained in 5 out of the 12 patients with advanced cancer so far treated in this manner. He is also seeking further significant genetic markers for drug sensitivity through DNA microarray technology.

Nobuyoshi Shimizu (Keio University, Tokyo) described disease gene hunting via genomic sequencing of human chromosomes 22 and 21. The Human Genome Project is approaching its goal: the complete sequencing of all human genes. His research team has been involved in the project by taking responsibility for analyzing several regions of human chromosomes 22 and 21 to find new genes which are associated with important biological and medical problems. Consequently, 545 and 225 genes were found, respectively, by means of combinations of several advanced molecular techniques such as exon trapping,

cDNA capture and genomic sequencing. A high-quality BAC library and chromosome-specific cosmid libraries were constructed and efficient PCR-based screening and high-fidelity digital hybridization methods were developed to construct BAC/cosmid DNA contigs. The shotgun sequencing method was utilized to completely determine the sequence with no gaps and high accuracy. The recovered genomic sequences are subjected to homology search and analysis of protein coding potential using various computer programs. Each candidate gene was confirmed as a disease-causing pathogenic gene by extensive mutational analysis of the patient's DNA. For chromosome 22, his team analyzed the peri-centromeric region of 7.1 Mb and identified over 45 genes including *CESK1*, *hSNF5/INI1*, *BCR*, *GGT*, *GGTR*, *GSTT*, *DDCT*, *BID* and *CECR1* and many pseudogenes. Particularly interesting genes include *CESK1* (t-complex protein-1 θ-like) which is located at the most centromeric region, and this together with *BID* (a regulatory subunit of calcium channel protein) and *CECR1* (a growth factor) appear to be candidate genes for cat eye syndrome. *hSNF5/INI1* seems to be responsible for rhabdoid tumor development. It is also noteworthy that 7 sites of *LCR22* (low copy repeat 22) consisting of *BCR* and *GGT* are strongly associated with the chromosomal breakpoints of certain diseases. For chromosome 21, four separate regions of 6.5 Mb were analyzed and 45 new genes and 15 pseudogenes were identified. These new genes include *C2lorf5*, *DSCR5*, *DSCR6*, *ZNF295*, *UMODL1*, *TMPRSS3*, *UBASH3A*, *TSGA2*, *SLC37A1*, *PDE9A*, *WDR4*, *SNF1LK*, *H2BSF*, *AGPAT3*, *TRPC7*, *AIRE*, *DNMT3L*, *SIM2*, *MNB* and 18 members of *KAP* gene family. *SIM2* (single-minded 2) encoding a PAS family transcription factor and *MNB* (minibrain) encoding a dual-specificity protein kinase were the first two genes discovered as candidate genes for the mental retardation of Down syndrome patients. *AIRE* (autoimmune regulator) encoding a transcription factor with SAND and PHD finger motifs was proven to be a pathogenic gene for autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *TRPC7* (transient receptor potential-related channels 7) encoding a putative Ca^{2+} channel transmembrane protein is considered to be a good candidate for bipolar affective disorder. *TMPRSS3* encoding a putative transmembrane serine protease was found to be responsible for the autosomal recessive nonsyndromic deafness DFNB10. Thus, his group has found a large number of new genes, although the physiological functions of the individual gene products still have to be established. However, it is obvious that many of these genes and their products are potential targets for biomedical studies which will ultimately contribute to the development of enhanced diagnostic and therapeutic regimens.

Hiroshi Shimada (Yokohama City University, Yokohama) discussed the gene expression profile of colorectal cancer patients, examined by cDNA microarray. Genome-wide information on gene expression in cancer cells promises to clarify many aspects of their clinical behavior. He and his colleague have begun a study of gene expression in colorectal cancers using around 21 000 human clones from Research Genetics Inc., which contain 4000 known genes and 16 000 other expression sequence tags. First, the expression profiles of colon adenocarcinomas were analyzed and compared to corresponding noncancerous mucosa samples. The fluorescence ratio of Cy3 (tumor) and Cy5 (normal) was scanned and quantified for each gene. For the analysis, two-way hierarchical clustering using the program Cluster (http://www.microarrays.org./software) was applied. A hierarchical clustering algorithm was used to group genes on the basis of similarity pattern in all samples. In the specimen dendrogram, the duplicates of replicated specimens clustered tightly together, indicating that the cluster is based on specific variations that are characteristic of these gene expression patterns. Increasing numbers of colorectal tumor cases are being analyzed in order to create a database of expression of genes involved in different histological types of colorectal cancer in relation to specific clinical background data of the patients. The cases were stratified with several clinical data, i.e. *p53* mutated- vs. wild-type, lymph node metastasis (+) vs. (−), liver metastasis (+) vs. (−), and the depth of tumor invasion m, sm vs. se, a2, etc. This strategy makes it possible to identify genes whose difference of expression is related to a specific phenomenon typical of colorectal cancer. Using this approach, 343 genes that could be associated with the depth of tumor invasion were extracted from the 20 000 starting clones. Although 83% of 343 genes belong to clones that at present have been classified as ESTs, several genes that are plausible candidates, i.e. HGF, TGF-β, collagenases etc., were found. The analysis of the expression pattern identified is still in progress. To predict high metastatic potential, 22 genes have been selected for diagnostic purposes and their clinical significance is being evaluated. cDNA microarrays will provide a huge amount of gene information. The final target for this technology from the clinical point of view is to find expression patterns associated with specific characteristics of subtypes of colorectal cancer, especially aggressive behaviors. The identification of genes regulating malignant progression will provide clues to develop better choices of therapy.

Hiroshi Yokozaki (Hiroshima University, Hiroshima) described a molecular-pathological diagnosis system using routine histopathology specimens. Multiple genetic and epigenetic alterations of cancer-related genes and molecules are involved in gastrointestinal carcinogenesis. These include telomerase activation, genetic instability and abnormalities of oncogenes, tumor suppressor genes, cell cycle regulators, cell adhesion molecules and DNA

repair genes. Alterations of *p53* and *APC* are good genetic markers for differential diagnosis. Gene amplification and overexpression of c-*met*, c-*erb*B2 TGFα, EGF receptor and cyclins are biological markers of malignancy. Microsatellite instability is an indicator for high susceptibility to cancers such as hereditary non-polyposis colorectal cancer, as well as for high risk for developing multiple cancers. Many of the genetic and epigenetic changes can be analyzed using paraffin-embedded pathology specimens. A system of molecular-pathological diagnosis was established and is available as a routine service in collaboration with Hiroshima City Medical Association Clinical Laboratory. Over 10 000 cases of gastrointestinal biopsy and surgery have been analyzed and reports of molecular diagnoses have been sent routinely to clinicians. New information relevant to differential diagnosis and biological malignancy, in addition to the pure histopathological diagnosis, could be obtained in about 15% of the cases by this molecular diagnosis strategy. Molecular-pathological diagnosis may provide a new approach to cancer diagnosis and novel therapeutics for the next century. Furthermore, analysis of the genetic and epigenetic abnormalities in clinical materials may clarify the molecular mechanisms of carcinogenesis and comparative morphological changes. Analysis of genetic instabilities indicated that the characteristic of gastric cancer with a high frequency of microsatellite instability is well differentiated histology, developed in aged patients. Molecular-pathological diagnosis can thus contribute to a detailed understanding of cancer histopathology and improve the histopathological diagnosis. This diagnostic approach may contribute to the new personalized medicine of the next century by characterizing cancer individually according to its genetic and epigenetic alterations. His group is planning to introduce DNA microarray technology into the routine system.

Yasuko Shirai (National Institute of Mental Health, Chiba) described ethical and psychosocial dilemmas of gene diagnosis. The rapid advance of the Human Genome Project has brought remarkable knowledge about genetic factors in disease, as discussed in this symposium. In addition, the development of technology for the detection of genetic disorders has enabled testing for such diseases with DNA techniques. With this progress in molecular genetics, it will be feasible to conduct presymptomatic testing for monogenic disorders of late onset, such as Huntington disease, and predictive testing of susceptibility to many common diseases with multifactorial causation, including both multiple genetic factors and environmental effects. Prediction of susceptibility to cancer by analyzing single nucleotide polymorphism of carcinogen-related enzymes is one possibility. Such gene diagnosis is consistent with the golden rule of medical practice, effective treatment may closely follow early diagnosis. At the same time, there are important ethical, legal and social implications (ELSI). She clearly described the benefits and harms of genetic testing and suggested that there are two distinct aspects: one is the nature of the genetic information of individual persons obtained by DNA testing and the other is the specific feature of predictive testing of susceptibility to common diseases. The following points should be considered separately in relation to these problems: (1) informed consent, (2) privacy and confidentiality, (3) conflict of interests within family members, (4) disclosure of personal genetic information without the consent of the person in question, (5) uncertainty of predictive testing, (6) stigmatization and discrimination based on genetic predisposition. She also referred to the importance of establishing a genetic counseling system in Japan.

Closing remarks were made by Eiichi Tahara (Hiroshima Cancer Seminar Foundation, Hiroshima). The seven presentations were summarized, and it was concluded that the analyses of genetic and epigenetic alterations by introducing new technology will give crucial information on individual characteristics of cancer, such as biological behavior and clinical outcome. He finally proposed to establish a new classification, TNM-G, including Genetic and epigenetic alterations in cancer, which will link directly with the new concept of personalized medicine.

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